Effects of Methionine Deficiencies on Plasma Levels of Thyroid Hormones, Insulin-like Growth Factors-I and -II, Liver and Body Weights, and Feed Intake in Growing Chickens

L. B. Carew,*†[1] J. P. McMurtry,‡ and F. A. Alster*

*Department of Animal Science and †Department of Nutrition and Food Science, University of Vermont, Burlington, Vermont 05405; and ‡USDA, ARS, Growth Biology Lab, Beltsville, Maryland 20705

ABSTRACT We showed previously that Met deficiency at 0.25% of the diet causes elevations in plasma triiodothyronine (T_3) in broilers. In the present study, plasma levels of thyroid hormones as well as insulin-like growth factors (IGF)-I and -II were measured in chicks fed 3 deficient levels of total Met. Control (0.5%) and Met-deficient diets (0.4, 0.3, and 0.2%) were fed to male broilers from 8 to 22 d of age. Additional groups of control chicks were pair-fed with the Met-deficient ones. Chicks receiving 0.4% Met increased feed intake by 10% with no significant change in body weight. The more severe Met deficiencies of 0.3 and 0.2% caused graded reductions in feed intake and weight gain. However, corresponding pair-fed control chicks were significantly heavier. These changes suggest more marked alterations in metabolic processes with 0.3 and 0.2% Met than with 0.4% Met. Liver weights were heavier in chicks fed 0.3 and 0.2% Met but not 0.4%. Plasma T_3 was higher in all deficient chicks compared with the free-fed control, which was significant only with 0.3% Met. However, with 0.3 and 0.2% Met, plasma T_3 was significantly elevated compared to pair-fed controls. Plasma thyroxine (T_4) was lower in all deficient groups, which was significant only with 0.2% Met, whereas no significant differences occurred between deficient chicks and their pair-fed controls. Plasma IGF-I levels were not significantly different, but they were consistently lower in deficient chicks and deserve further study. Plasma IGF-II was significantly less in chicks fed 0.2% Met compared to pair-fed controls suggesting that Met deficiency interferes with IGF-II metabolism. We concluded that a deficit of dietary Met altered plasma T_3 and IGF-II levels, but the effect was dependent on the degree of deficiency.

(*Key words*: broiler chick, insulin-like growth factor, liver weight, methionine deficiency, thyroid hormone)

2003 Poultry Science 82:1932–1938

INTRODUCTION

In regions where soybeans are the primary proteinaceous component of poultry rations, Met is the first limiting essential amino acid (EAA). Its need in poultry rations for growth has been studied extensively (Wicker, 1990; NRC, 1994), although there is considerable disagreement as to factors that influence its dietary requirement (NRC, 1994). Many aspects of a Met deficiency on avian metabolism have been studied such as its interaction with choline, betaine, folic acid, and vitamin B_{12} as well as effects on the immune system. More extensive studies in mammalian species have been reviewed (Finkelstein, 1990).

Very few studies have been done on the effects of Met deficiency on the avian endocrine system. Carew et al. (1997) reported that plasma levels of triiodothyronine (T_3) were elevated in 3-wk-old broilers given 50% of the Met requirement (0.25% of diet) compared with pair-fed controls given equal amounts of feed. Thyroxine (T_4) was not significantly changed. This experimental design took into account the depressive effects of the Met deficiency on feed intake. Deficiencies of Arg, Lys, and Ile but not other EAA showed similar effects. Therefore, earlier results showing that T_3 is elevated in protein-deficient chicks (Alster and Carew, 1984; Keagy et al., 1987) could not be explained by a common effect of individual EAA deficiencies on thyroid hormone metabolism.

Insulin-like growth factors (IGF) have not been studied in Met-deficient chickens. Calorie and protein deficiencies, however, do have an effect on these hormones, albeit differential. Chickens consuming a low protein diet exhibit depressed plasma IGF-I levels, which return to normal on restoration of dietary protein (Lauterio

Received for publication April 2, 2003.
Accepted for publication August 27, 2003.
[1]To whom correspondence should be addressed: lyndon.carew@uvm.edu.

Abbreviation Key: EAA = essential amino acid; IGF = insulin-like growth factor; IGFBP = insulin-like growth factor binding protein; T_3 = triiodothyronine; T_4 = thyroxine.

and Scanes, 1988; Rosebrough et al., 1989; Rosebrough and McMurtry, 1993). Feeding low protein diets, however, does not alter tissue IGF-I mRNA activity (Kita et al., 1996). During short-term fasting, serum or plasma levels of IGF-I are depressed and remain low on refeeding (Kim et al., 1991; Beccavin et al., 2001) or return totally to normal (Kita et al., 1996). In addition, IGF-I mRNA is depressed during fasting (Beccavin et al., 2001). IGF-II has been reported to decrease (Beccavin et al., 2001) or increase (McMurtry, 1998) during feed withdrawal but is restored to normal on refeeding. IGF-I seems to be more sensitive to dietary restriction than IGF-II, and dietary manipulation can variably affect the various IGF binding proteins (IGFBP) (Leili and Scanes, 1998; Beccavin et al., 1999; Caperna et al., 1999; Kita et al., 2002).

In early work with rats (Bolze et al., 1985), Met deficiency was shown to reduce serum somatomedin (measured using the embryonic chick pelvic rudiment method). More recently, protein deficiency (see reviews by Thissen et al., 1994; Ketelslegers et al., 1995) and Met deficiency have been shown to depress plasma IGF-I levels in rats but to have no effect on hepatic IGFBP-1 mRNA content or plasma levels of IGFBP-1 (Ammann et al., 2000; Takenaka et al., 2000).

In our earlier work only the effect of severe Met deficiency (0.25% of the diet) on thyroid function in growing chicks was measured. Therefore, the purpose of the present experiment was to determine the effect of borderline and marked Met deficiencies on thyroid function and to include IGF hormones as added parameters.

MATERIALS AND METHODS

Bird Management

One-day-old male broiler chicks[2] (175) were housed in electrically heated, starter battery brooders with raised wire floors and internal lighting. Birds were fed a control diet complete in all nutrients including EAA (Table 1). At 8 d of age the chicks were sorted into groups with weight ranges of 3 to 5 g. They were then evenly distributed into 21 pens of 7 chicks each (147 total chicks). High and low weight extremes were eliminated. Triplicate groups of chicks were used per treatment. Initial pen weights at 8 d were similar for all 21 pens (188 ± 1 g). The treatments were a control (0.5% total Met) and 3 deficient levels of total Met (0.4, 0.3, and 0.2%).

Groups of control chicks were pair-fed with deficient chicks by matching a pen of chicks fed the control diet with one of the deficient pens. Control feed was provided once daily to the pair-fed group at 0800 h, which was the same amount as eaten by the Met-deficient chicks the previous day. This method was followed to account for the effect of differences in feed intake between the control group given free access and the deficient groups. Brooder temperatures were wk 1, 35°C; wk 2, 32°C; and wk 3, 29°C. Room temperature was maintained between 23 and 26°C. Water was given freely and a cycle of 16L:8D was used. The experimental period lasted 2 wk, from 8 to 22 d of age.

TABLE 1. Composition of broiler starter diet[1]

Ingredient	%
Yellow corn meal	51.07
Soybean meal (dehulled)	35.40
Soybean oil	4.00
Poultry blend meal	3.00
Alfalfa meal	1.00
Distiller's dried grains	2.00
Dicalcium phosphate	1.30
Limestone	1.10
Salt	0.40
Vitamin mix[2]	0.50
Trace mineral mix[3]	0.10
DL-Methionine	0.13

[1]Contained (by calculation) 24.4% protein and 3,148 kcal/kg metabolizable energy. As percentages, arginine, 1.60; cystine, 0.47; glycine + serine, 3.02; histidine, 0.63; isoleucine, 1.00; lysine, 1.31; methionine, 0.51; phenylalanine, 1.15; threonine, 0.90; tryptophan, 0.28; tyrosine, 0.94; valine, 1.13.

[2]Supplied (mg/kg diet) riboflavin, 4.4; D-pantothenic acid Ca-salt, 8.8; niacin, 20; choline, 322; menadione, 2; vitamin E, 11; vitamin B_{12}, 0.01; ethoxyquin, 33; and vitamin A 5,200 IU/kg; vitamin D_3 1,000 IU/kg.

[3]Supplied (mg/kg diet) Fe, 40, as $FeSO_4 \cdot 7H_2O$; Mn, 75, as $MnSO_4 \cdot H_2O$; Zn, 60, as ZnO; Cu, 4, as $CuSO_4 \cdot 5H_2O$; I, 0.86, as KIO_3; Se, 0.10, as Na_2SeO_3; Ca, 136, as $CaCO_3$.

Diets and Experimental Design

The nutritionally complete control diet used during the 14-d experiment consisted of a 50:50 mixture of a broiler starter diet (Table 1) and a purified amino acid diet (Table 2) slightly modified from Scott et al. (1982). It contained 24% protein and the NRC (1994) recommended level of 0.5% total Met. Methionine-deficient diets (0.4, 0.3, and 0.2% total Met) were formulated by deleting appropriate amounts of Met from the purified portion of the mixed diet and replacing it with sucrose. This type of diet was advantageous, as it would allow for the study of other amino acid deficiencies using the same control diet.

Blood Samples, Hormone Assays, and Statistics

At 22 d, blood was drawn from each chick by side heart puncture between 1000 and 1300 h and placed into heparinized tubes. The tubes were centrifuged at 1,800 × *g* for 15 min to obtain plasma, which was frozen for later analysis. Plasma total T_3 and T_4 levels were analyzed by RIA,[3] which had been validated as described previously (Carew et al., 1997). Specificity of the assays for T_3 and T_4 had been established by the supplier. Intra- and interassay CV were 6.04 and 9.71% for T_3 and 13.29 and 7.80% for T_4, respectively. IGF-I and -II were analyzed by homologous RIA (McMurtry et al., 1994, 1997).

[2]Hubbard Farms, Walpole, NH.
[3]Clinical Assays, Cambridge, MA.

TABLE 2. Composition of purified amino acid diet[1]

Ingredient	%
Sucrose	59.377
Amino acid mix[2]	23.640
Mineral mix[3]	8.270
Soybean oil	5.000
Cellulose (Solka-Floc)[4]	3.000
Vitamin mix[5]	0.500
Choline chloride (70%)	0.200
Ethoxyquin[6]	0.013

[1]Equivalent to 24% protein.

[2]Supplied (L-isomers except methionine and glycine) as percentage of diet: tryptophan, 0.22; histidine HCl, 0.41; tyrosine, 0.63; phenylalanine, 0.68; methionine, 0.55; cystine, 0.35; threonine, 0.65; leucine, 1.20; isoleucine, 0.80; valine, 0.82; glycine, 1.60; proline, 1.00; lysine HCl, 1.40; arginine HCl, 1.33; glutamic acid, 12.00.

[3]Supplied (g/kg diet) $CaHPO_4$, 27; $CaCO_3$, 11; $NaHCO_3$, 15; KH_2PO_4, 9; NaCl, 8.8; $MnSO_4 \cdot H_2O$, 0.70; $FeSO_4 \cdot 7H_20$, 0.5; $MgSO_4 \cdot H_2O$, 3.5; KIO_3, 0.0025; $CuSO_4 \cdot 5H_2O$, 0.03; H_3BO_3, 0.009; ZnO, 0.105; $CoCl_3 \cdot 6H_2O$, 0.002; $NaMoO_4 \cdot 2H_2O$, 0.009; Na_2SeO_3, 0.0002.

[4]Brown Co., Berlin, NH.

[5]Supplied (mg/kg diet) riboflavin, 15; D-pantothenic acid Ca-salt 50; niacin, 100; pyridoxine, HCl, 15; thiamin, HCl, 15; folacin, 6; biotin, 0.5; vitamin B_{12}, 0.05; menadione Na bisulfite (63 to 75%), 5; DL-α-tocopheryl acetate (250 IU/g), 200; vitamin A (500,000 IU/g), 19.5; cholecalciferol (1,000,000 IU/g), 4.8.

[6]1,2′-dihydro-6-ethoxy-2,2,4- trimethylquinoline, Solutia, St. Louis, MO.

Intra- and interassay CV were 2.39 and 4.67% for IGF-I and 3.02 and 4.89% for IGF-II, respectively.

Data were analyzed using the general linear models ANOVA with $P \leq 0.05$ considered significant (SAS, 1990). Means were compared with Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

When total dietary Met was fed at levels below the NRC (1994) recommendation of 0.5% (0.4, 0.3, and 0.2%), a significant decrease in feed intake was observed only with 0.3 and 0.2% Met, and the latter was the most severe (Table 3). To the contrary with the mild deficiency of 0.4%, there was a significant increase in feed intake compared with the free-fed control. Weight gain was not affected until the Met level reached 0.3% or less (Table 3). The exact requirement for Met in relation to that set by the NRC (1994) at 0.5% is open to considerable question. As discussed in the NRC (1994) report, of the studies done on Met requirements of broilers 0 to 21 d of age, 2 research groups found it to be higher, 4 found it to be about the same, and 2 found it to be lower. Our results during the 8- to 22-d period suggest that 0.4% dietary Met is sufficient for optimum growth. However, we interpret the small but significant increase in feed intake by chicks fed 0.4% Met as an indication that this level is still deficient, and chicks compensate for the deficiency by overeating. Therefore, our observations suggest that for the 8- to 22-d period with the diet used, a dietary level greater than 0.4% Met is needed and approaches the listed NRC requirement of 0.5% Met for chicks 0 to 3 wk of age.

An increase in food intake in the absence of a change in weight gain by chicks fed a borderline deficiency of Met confirms earlier results (Carew and Hill, 1961; Sekiz et al., 1975). Then and now, this finding is interpreted to mean that chicks marginally deficient in Met overeat slightly to consume the adequate amounts of Met needed. This increased feed intake does not cause an increase in weight gain because the added caloric intake is converted to body fat, which replaces body water (Carew and Hill, 1961). Thus, with a borderline Met deficiency of 0.4%, feed intake is increased, but body protein synthesis is decreased, and the surplus calories are shunted into fat synthesis. These changes are not a consequence of alterations in energy utilization due to changes in metabolizable energy value of the diet (i.e., absorbability) or in the efficiency of energy processing (Carew and Hill, 1961). A similar situation exists when there is a marginal protein deficiency in growing broilers (Keagy et al., 1987).

Comparisons of growth data between chicks more deficient in Met (0.3 and 0.2%) and their matched, pair-fed controls (Table 3) strongly suggest that marked changes in metabolism had occurred. At these 2 dietary levels of Met, matched chicks pair-fed the control diet gained much more weight than the deficient chicks. This could be a result of further increases in fat synthesis at the expense of body protein synthesis in the deficient

TABLE 3. Feed intake, growth rate and liver weights of male broilers deficient in methionine[1]

Dietary methionine treatment	Feed intake (g)	Weight gain (g)	Feed efficiency (gain/feed)	Liver (g)	Liver (g/kg of BW)
Control 0.5% free-fed	920^{bc}	693^{a}	0.75^{a}	27.6^{a}	29.6^{c}
Deficient 0.4% free-fed	1,004^{a}	734^{a}	0.73^{ab}	26.5^{a}	29.2^{c}
Control 0.5% pair-fed	938^{ab}	697^{a}	0.74^{a}	26.3^{a}	30.2^{bc}
Deficient 0.3% free-fed	851^{c}	513^{c}	0.60^{c}	23.9^{b}	32.9^{ab}
Control 0.5% pair-fed	846^{c}	596^{b}	0.70^{c}	23.9^{b}	31.3^{abc}
Deficient 0.2% free-fed	448^{d}	179^{e}	0.40^{d}	12.6^{c}	34.1^{a}
Control 0.5% pair-fed	488^{d}	293^{d}	0.60^{c}	12.3^{c}	26.2^{d}
Pooled standard deviation	44	34	0.02	3.1	3.4

[a–e]Means within a column lacking a common superscript are significantly different ($P < 0.05$).

[1]The experimental period was 8 to 22 d of age. Feed and weight gain data represent triplicate groups of 7 chicks each. Liver data represent 12 observations per treatment.

TABLE 4. Blood plasma levels of thyroid hormones and insulin-like growth factors (IGF) in male broilers deficient in methionine[1]

Dietary methionine treatment	T_3 (ng/mL)	T_4 (μg/dL)	IGF-I (ng/mL)	IGF-II (ng/mL)
Control 0.5% free-fed	2.82^{bc}	3.98^{a}	36.7^{a}	29.9^{b}
Deficient 0.4% free-fed	3.28^{ab}	3.68^{ab}	39.8^{a}	32.0^{ab}
Control 0.5% pair-fed	2.85^{bc}	3.74^{ab}	36.6^{a}	30.8^{b}
Deficient 0.3% free-fed	3.59^{a}	3.63^{ab}	32.2^{a}	34.5^{ab}
Control 0.5% pair-fed	2.97^{b}	3.73^{ab}	37.2^{a}	34.5^{ab}
Deficient 0.2% free-fed	3.21^{ab}	3.58^{b}	25.0^{a}	31.0^{b}
Control 0.5% pair-fed	2.40^{c}	3.97^{ab}	33.7^{a}	39.8^{a}
Pooled standard deviation	0.61	0.48	27.9	10.8

[a–c]Means within a column lacking a common superscript are significantly different ($P < 0.05$).

[1]The experimental period was 8 to 22 d of age. Each value is the average of 14 to 15 samples taken at 21 d of age.

chicks, as we have suggested with 0.4% Met, and which is supported by our earlier work. However, increases in catabolism and heat loss cannot be excluded. Sekiz et al. (1975) observed increased heat production in chicks fed a severely deficient level of 0.25% Met but not at 0.32%.

The overall result of these changes in feed intake and weight gain was that the ratio of weight gain to feed intake decreased significantly only with 0.3 and 0.2% Met (Table 3), and it was significantly worse with 0.2% Met.

Changes in liver weight are often an index of various nutrient deficiencies, including protein. We found that average liver weight was not altered at the dietary level of 0.4% Met but decreased with 0.3 and 0.2% Met, very markedly in the latter case (Table 3). However, when expressed in relation to BW (relative weight), liver weights were not changed at 0.4% Met but increased with the more severe deficiencies. There was no significant difference between 0.3 and 0.2% Met.

On the other hand, chicks fed the control diet but given the same amount of feed as the deficient chicks (i.e., pair-fed) showed no change from the free-fed controls (0.3% Met) or actually showed a decrease in liver weight relative to BW. This finding means that reduced feed intake actually causes no change or causes a reduction in relative liver weights, whereas Met deficiency causes an enlargement. This result demonstrates that Met deficiency has a specific effect on relative liver growth apart from any effect that can be attributed to the depressive effect of Met deficiency on feed intake.

A deficiency of a balanced mixture of EAA produces the same effect and can be attributed, in part, to an accumulation of liver fat (Velu et al., 1971). Fatty livers seem to be a common occurrence in chicks and other animals fed deficient levels of protein (Velu et al., 1971; Keagy et al., 1987). Therefore, like a protein deficiency, a Met deficiency causes an increase in liver size probably due to an increase in fat content, although that was not measured in this study.

Plasma T_3 in the Met-deficient chicks showed an inverted U relationship compared to the free-fed control (Table 4). It was not significantly different at 0.4% Met, increased significantly at 0.3% Met, but then decreased to control level again as Met declined further to 0.2%. This result agrees with our earlier results that showed elevated plasma T_3 with a single Met deficiency at 0.25% of the diet (50 % of the NRC 1994 recommendation) and further shows that when the deficiency is even more severe, plasma T_3 will return to the level in the free-fed control chicks. Of greater importance, however, are the plasma T_3 comparisons between deficient chicks and their matched, pair-fed controls. We found no significant difference at the 0.4 % Met level, but with 0.3 and 0.2% Met, plasma T_3 levels were significantly higher than in their pair-fed controls. This comparison is proper as it takes into account any effects of reduced feed intake on plasma T_3 values.

It has been shown in several species including chickens (Alster and Carew, 1984; Keagy et al., 1987) and rats (Glass et al., 1978; Tyzbir et al., 1981) that plasma T_3 will decrease in response to restricted feed intake or fasting. We observed this decrease in the present study with the most severe Met deficiency. Thus when properly compared with control chicks fed the same reduced amount of feed, plasma T_3 is always higher in deficient chicks (i.e., it does not decrease as much as expected on the basis of reduced feed intake alone). We interpret this finding to mean that the Met deficiency increases the production or release of T_3 into the blood or inhibits its normal removal compared with control chicks consuming the same amount of feed. This may operate through inhibited synthesis of a key protein involved in the metabolism or turnover of T_3 due to lack of sufficient Met for polypeptide synthesis. From studies reported with protein deficiencies in chicks or rats, it has been suggested that elevated T_3 may be a consequence of increased secretion rate and activity of the thyroid gland (March et al., 1964; Tulp et al., 1979), slower clearance of T_3 from the blood (Hutchins and Newcomber, 1966), or alterations in plasma-binding capacity of the blood and changes in receptor binding or affinity (Refetoff et al., 1970; Smallridge et al., 1982; Rouaze-Romet et al., 1992), among others. Enhanced conversion of T_4 to T_3 due to increased hepatic or renal 5′-deiodinase activ-

ity does not seem to occur with a protein deficiency in rats (Smallridge et al., 1982) or chicks (Weyland, 1993). However, in the absence of direct data with Met and in view of many other roles of Met in animals (Finkelstein et al., 1982; Hawrylewicz and Huang, 1992), other mechanisms of an unknown nature may be involved. Nevertheless, based on the T_3 data, it is certain that Met deficiency alters normal thyroid hormone metabolism.

Plasma T_4 was minimally affected by the Met deficiency (Table 4). Compared with free-fed control chicks, T_4 was significantly lower in those fed the most deficient level of 0.2% Met, but there was no significant difference between these chicks and their pair-fed controls. Thus, the possibility exists that the lower T_4 in chicks fed 0.2% dietary Met is simply a consequence of a reduction in feed intake. This finding agrees with our earlier results when a single deficient level of 0.25% was fed (Carew et al., 1997).

Plasma IGF-I levels were not significantly altered by Met deficiency or reduced feed intake (Table 4). The lack of effect of lower feed intake on plasma IGF levels in the pair-fed chicks on restricted levels of the control diet is contrary to observations by others who reported a decrease in blood IGF-I during fasting (Kim et al., 1991; Kita et al., 1996; Beccavin et al., 2001). However, fasting conditions are very different from the situation in this study (fed state), which may indicate that some minimal level of feed intake is sufficient to maintain near normal circulating IGF-I levels. It is also possible that small differences in the balance of EAA between commercial-type diets used by others and our 50:50 mix of commercial-type and purified diets played a role.

Although the balance of EAA in both types of diets is adequate for full-fed chicks, little is known about the balance of EAA needed for optimal performance under conditions of restricted feeding. No studies of plasma IGF have been reported in Met-deficient chicks before the present study. Although there were no significant differences in IGF-I values between chicks fed the control diet and Met-deficient chicks, numerically IGF-I values for the deficient chicks were lower. Because Met deficiency in rats causes a reduction in plasma IGF-I values (Takenaka et al., 2000), our results, although not significant, suggest that further study of this relationship should be done.

Plasma IGF-II values of control chicks pair-fed with the 0.2% Met-deficient chicks were significantly higher than the free-fed controls (Table 4). None of the other IGF-II values was significantly different from the free-fed control. The only other significant difference was between the 0.2% Met group and its pair-fed control, with the Met-deficient values being lower. IGF-II has been reported to decrease (Beccavin et al., 2001) or increase (McMurtry et al., 1998) during feed withdrawal. Our observation agrees with the latter. That IGF-II levels in deficient chicks fed 0.2% Met did not rise along with the matched, pair-fed controls, even though they consumed the same reduced level of feed, suggests the Met deficiency interfered with the metabolism of IGF-II in the blood. The mechanism of this effect, whether this reflects increased production of IGF-II or decreased removal, is unknown. As far as we know, this study was the first on the relationship between a Met deficiency and blood IGF-II levels, and the differences shown suggest that further studies are in order.

The question arises as to interactions between circulating levels of thyroid hormones and IGF-I, which have been previously studied in chickens. It is well established that elevated plasma T_3 reduces plasma growth hormone levels in chickens, (see reviews by Scanes, 1987; Harvey et al., 1991). Nevertheless, research also shows that thyroidal inhibition of growth hormone is not related to changes in IGF-I (Lauterio and Scanes, 1988), and treatment of chicks with T_3 or T_4 has no effect on levels of plasma IGF-I (Lazarus and Scanes, 1988). In the present study, changes in circulating levels of T_3 or T_4 did not alter plasma IGF-I levels. With the highest or lowest T_3 levels (3.59 ng/mL in the 0.3% Met treatment or 2.40 ng/mL in the controls pair-fed to the 0.2% Met treatment, Table 4), IGF-I levels were unchanged. With regards to T_4, although the lowest plasma level was associated with the lowest IGF-I concentration (3.58 and 25.0 ng/mL, respectively, in the 0.2% Met treatment), no other interaction was apparent. Thus there is no evidence in this study of a relationship between changes in plasma T_3 or T_4 levels and circulating levels of IGF-I.

Interactions between blood levels of thyroid hormones and IGF-II in chickens have not been reported. The relevance of IGF-II compared with IGF-I is open to question, as no unique IGF-II receptor has been found in birds (McMurtry et al., 1997). Nevertheless, the lowest plasma T_3 value found in chicks fed the most severely restricted amount of the control diet (matched to the 0.2% Met treatment) was associated with the significantly highest IGF-II value in the same treatment (Table 4). Severe feed restriction in chicks has been reported to depress plasma T_3 values (Alster and Carew, 1984; Keagy et al., 1987) as well as increase plasma IGF-II values (McMurtry et al., 1998). A direct comparison of the two in a single study has not been reported. Therefore, our results, showing this inverse relationship when measured in the same chicks, suggest interactive effects between the 2 hormones and deserves further study.

ACKNOWLEDGMENTS

The authors appreciate the generous donation of chicks by Hubbard Farms and the donation of materials from International Minerals and Chemicals Corp., Monsanto Co., Hoffmann-LaRoche, Inc., Merck & Co., Morton Salt Co., Pitman-Moore Inc., and Chas. Pfizer Co., Inc. This research was supported by a grant from the USDA and from the Committee on Research and Scholarship, University of Vermont. The help of the staff in the Department of Animal Science is greatly appreciated.

REFERENCES

Alster, F. A., and L. B. Carew, Jr. 1984. Studies of energy balance and thyroid function in protein-deficient chicks. Nutr. Rep. Int. 30:1231–1240.

Ammann, P., S. Bourrin, J. P. Bonjour, J. M. Meyer, and R. Rizzoli. 2000. Protein undernutrition-induced bone loss is associated with decreased IGF-I levels and estrogen deficiency. J. Bone Miner. Res. 15:683–690.

Beccavin, C., B. Chevalier, L. A. Cogburn, J. Simon, and M. J. Duclos. 2001. Insulin-like growth factors and body growth in chickens divergently selected for high or low growth rate. J. Endocrinol. 168:297–306.

Beccavin, C., B. Chevalier, J. Simon, and M. J. Duclos. 1999. Circulating insulin-like growth factors (IGF-I and -II) and IGF binding proteins in divergently selected fat or lean chickens: effect of prolonged fasting. Growth Horm. IGF Res. 9:187–194.

Bolze, M. S., R. D. Reeves, F. E. Lindbeck, and M. J. Elders. 1985. Influence of selected amino acid deficiencies on somatomedin, growth and glycosaminoglycan metabolism in weanling rats. J. Nutr. 115:782–787.

Caperna, T. J., R. W. Rosebrough, J. P. McMurtry, and R. Vasilatos-Younken. 1999. Influence of dietary protein on insulin-like growth factor binding proteins in the chicken. Comp. Biochem. Physiol. 124B:417–421.

Carew, L. B., K. G. Evarts, and F. A. Alster. 1997. Growth and plasma thyroid hormone concentrations of chicks fed diets deficient in essential amino acids. Poult. Sci. 76:1398–1404.

Carew, L. B., Jr., and F. W. Hill. 1961. The effect of methionine deficiency on the utilization of energy by the chick. J. Nutr. 74:185–190.

Duncan, D. B. 1955. Multiple range and multiple *F* tests. Biometrics 11:1–42.

Finkelstein, J. D. 1990. Methionine metabolism in mammals. J. Nutr. Biochem. 1:228–237.

Finkelstein, J. D., W. E. Kyle, B. J. Harris, and J. J. Martin. 1982. Methionine metabolism in mammals: concentration of metabolites in rat tissues. J. Nutr. 112:1011–1018.

Glass, A. R., R. Mellit, K. D. Burman, L. Wartofsky, and R. S. Swerdloff. 1978. Serum triiodothyronine in undernourished rats: Dependence on dietary composition rather than total calorie or protein intake. Endocrinology 102:1925–1928.

Harvey, S., R. A. Fraser, and R. W. Lea. 1991. Growth hormone secretion in poultry. Crit. Rev. Poult. Biol. 3:239–282.

Hawrylewicz, E. J., and H. H. Huang. 1992. Effects of dietary protein and methionine supplementation on mammary tumorigenesis. Pages 139–148 in Dietary Proteins. How They Alleviate Disease and Promote Better Health. G. U. Liepa, ed. Am. Oil Chemists Society, Champaign, IL.

Hutchins, M. O., and W. S. Newcomber. 1966. Metabolism and excretion of thyroxine and triiodothyronine in chickens. Gen. Comp. Endocrinol. 6:239–248.

Keagy, E. M., L. B. Carew, F. A. Alster, and R. S. Tyzbir. 1987. Thyroid function, energy balance, body composition and organ growth in protein-deficient chicks. J. Nutr. 117:1532–1540.

Ketelslegers, J., D. M. Maiter, M. Maes, L. E. Underwood, and J. P Thissen. 1995. Nutritional regulation of insulin-like growth factor-I. Metabolism 44:50–57.

Kim, J. W., D. L. Fletcher, D. R. Campion, H. R. Gaskins, and R. Dean. 1991. Effect of dietary manipulation on c-myc RNA expression in adipose tissue, muscle and liver of broiler chickens. Biochem. Biophys. Res. Comm. 180:1–7.

Kita, K., K. Nagao, N. Taneda, Y. Inagaki, K. Hirano, T. Shibata, M. A. Yaman, M. A. Conlon, and J. Okumura. 2002. Insulin-like growth factor binding protein-2 gene expression can be regulated by diet manipulation in several tissues of young chickens. J. Nutr. 132:145–151.

Kita, K., F. M. Tomas, P. C. Owens, S. E. Knowles, B. E. Forbes, Z. Upton, R. Hughes, and F. J. Ballard. 1996. Influence of nutrition on hepatic IGF-I mRNA levels and plasma concentrations of IGF-I and IGF-II in meat-type chickens. J. Endocrinol. 149:181–190.

Lauterio, T. J., and C. G. Scanes. 1988. The role of thyroid hormones in the growth hormone response to protein restriction in the domestic fowl (*Gallus domesticus*). J. Endocrinol. 117:223–228.

Lazarus, D. D., and C. G. Scanes. 1988. Acute effects of hypophysectomy and administration of pancreatic and thyroid hormones on circulating concentrations of somatomedin-C in young chickens: Relationship between growth hormone and somatomedin-C. Domest. Anim. Endocrinol. 5:283–289.

Leili, S., and C. G. Scanes. 1998. The effects of protein restriction on insulin-like growth factor-I and IGF-binding proteins in chickens. Proc. Soc. Exp. Biol. Med. 218:322–328.

March, B. E., J. Biely, and K. R. Pastro. 1964. The effect of protein level and amino acid balance upon thyroid activity in the chick. Can. J. Biochem. 42:341–344.

McMurtry, J. P. 1998. Nutritional and developmental roles of insulin-like growth factors in poultry. J. Nutr. 128:302S–305S.

McMurtry, J. P., G. L. Francis, and Z. Upton. 1997. Insulin-like growth factors in poultry. Domest. Anim. Endocrinol. 14:199–229.

McMurtry, J. P., G. L. Francis, F. Z. Upton, G. Rosselot, and D. M. Brocht. 1994. Developmental changes in chicken and turkey insulin-like growth factor-I (IGF-I) studied with a homologous radioimmunoassay for chicken IGF-I. J. Endocrinol. 142:225–234.

McMurtry, J. P., R. W. Rosebrough, D. M. Brocht, G. L. Francis, Z. Upton, and P. Phelps. 1998. Assessment of developmental changes in chicken and turkey insulin-like growth factor-II by homologous radioimmunoassay. J. Endocrinol. 157:463–473.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

Refetoff, S., N. I. Robin, and V. S. Fang. 1970. Parameters of thyroid function in serum of 16 selected vertebrate species: A study of PBI, serum T_4, free T_4, and the pattern of T_4 and T_3 binding to serum proteins. Endocrinology 86:793–805.

Rosebrough, R. W., and J. P. McMurtry. 1993. Protein and energy relationships in the broiler chicken. Effects of protein quantity and quality on metabolism. Brit. J. Nutr. 70:667–678.

Rosebrough, R., J. McMurtry, J. Proudman, and N. Steele. 1989. Comparison between constant-protein, calorie-restricted and protein-restricted, calorie-restricted diets on growth, in vitro lipogenesis and plasma growth hormone, thyroxine, triiodothyronine and somatomedin-C (Sm-C) of young chickens. Comp. Biochem. Physiol. 93A:337–343.

Rouaze-Romet, M., L. Savu, R. Vranckx, F. Bleiberg-Daniel, B. Le Moullac, P. Gouache, and E. A. Nunez. 1992. Re-expression of thyroxine-binding globulin in post-weaning rats during protein or energy malnutrition. Acta Endocrinol. 127:441–448.

SAS Institute. 1990. SAS/STAT User's Guide. Version 6. 4th ed. Vol. 2. SAS Institute Inc., Cary, NC.

Scanes, C. G. 1987. The physiology of growth, growth hormone, and other growth factors in poultry. Crit. Rev. Poult. Biol. 1:51–105.

Scott, M. L., M. C. Nesheim, and R. J. Young. 1982. Methods for chemical and biological measurement of nutritive quality. Pages 545–547 in Nutrition of the Chicken. 3rd ed. M. L. Scott & Assoc., Ithaca, NY.

Sekiz, S. S., M. L. Scott, and M. C. Nesheim. 1975. The effect of methionine deficiency on body weight, food and energy utilization in the chick. Poult. Sci. 54:1184–1188.

Smallridge, R. C., A. R. Glass, L. Wartofsky, K. R. Latham, and K. D. Burman. 1982. Investigations into the etiology

of elevated serum T_3 levels in protein-malnourished rats. Metabolism 31:538–542.
Takenaka, A., N. Oki, S. Takahashi, and T. Noguchi. 2000. Dietary restriction of single essential amino acids reduces plasma insulin-like growth factor-1 (IGF-1) but does not affect plasma IGF-binding protein-1 in rats. J. Nutr. 130:2910–2914.
Thissen, J., J. Ketelslegers, and L. E. Underwood. 1994. Nutritional regulation of the insulin-like growth factors. Endocr. Rev. 15:80–101.
Tulp, O. L., P. P. Krupp, E. Danforth, Jr., and E. S. Horton. 1979. Characteristics of thyroid function in experimental protein malnutrition. J. Nutr. 109:1321–1331.
Tyzbir, R. S., A. S. Kunin, N. M. Sims, and E. Danforth. 1981. Influence of diet composition on serum triiodothyronine (T3) concentration, hepatic mitochondrial metabolism and shuttle system activity in rats. J. Nutr. 111:252–259.
Velu, J. G., D. H. Baker, and H. M. Scott. 1971. Protein and energy utilization by chicks fed graded levels of a balanced mixture of crystalline amino acids. J. Nutr. 101:1249–1256.
Weyland, C. E. 1993. The effect of protein deficiency on plasma thyroid hormone levels, and on hepatic and renal 5′-deiodinase activity in broiler chickens. M.S. Thesis. University of Vermont, Burlington, VT.
Wicker, D. L. 1990. Methionine's past. Feed Manage. 41:64–66.